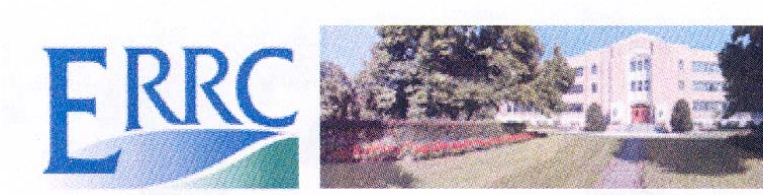

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Relationship of Cell Surface Charge and Hydrophobicity to Strength of Attachment of Bacteria to Cantaloupe Rind†

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ABSTRACT

The cantaloupe melon has been associated with outbreaks of *Salmonella* infections. It is suspected that bacterial surface charge and hydrophobicity may affect bacterial attachment and complicate bacterial detachment from cantaloupe surfaces. The surface charge and hydrophobicity of strains of *Salmonella, Escherichia coli* (O157:H7 and non-O157:H7), and *Listeria monocytogenes* were determined by electrostatic and hydrophobic interaction chromatography, respectively. Initial bacterial attachment to cantaloupe surfaces and the ability of bacteria to resist removal by washing with water were compared with surface charge and hydrophobicity. Whole cantaloupes were submerged in inocula containing individual strains or in cocktails containing *Salmonella, E. coli,* and *L. monocytogenes,* either as a mixture of strains containing all three genera or as a mixture of strains belonging to a single genus, for 10 min. Inoculated cantaloupes were dried for 1 h in a biosafety cabinet and then stored for up to 7 days at 4°C. Inoculated melons were washed with water, and bacteria still attached to the melon surface, as well as those in the wash water, were enumerated. Initial bacterial attachment was highest for individual strains of *E. coli* and lowest for *L. monocytogenes,* but *Salmonella* exhibited the strongest attachment on days 0, 3, and 7. When mixed-genus cocktails were used, the relative degrees of attachment of the three genera ware altered. The attachment of *Salmonella* strains was the strongest, but the attachment of *E. coli* was more extensive than that of *L. monocytogenes* on days 0, 3, and 7. There was a linear correlation between bacterial cell surface hydrophobicity (r^2 = 0.767), negative charge (r^2 = 0.738), and positive charge (r^2 = 0.724) and the strength of bacterial attachment to cantaloupe surfaces.

The ability of pathogenic bacteria to adhere to surfaces of fruits and vegetables continues to be a potential food safety problem of great concern to the produce industry. Surface structure and the biochemical characteristics of bacteria and of a substratum play a major role in how and where bacteria may attach. The surface of the cantaloupe is composed of a meshwork of tissue commonly referred to as the *net (44).* The raised net tissue gives the surface of the cantaloupe an inherent roughness. The surface roughness may favor microbial attachment and hinder microbial detachment.

Bacterial attachment to surfaces is influenced not only by cell surface charge *(14)* and hydrophobicity *(41–43)* but also by the presence of particular surface appendages such as flagella and fimbriae, as well as extracellular polysaccharides *(13, 15).* Flagella, fimbriae (pili), outer membrane proteins, and extracellular polysaccharides may influence bacterial attachment to plant surfaces *(30, 36).* Plant surfaces and microbes both have negative surface potential, which results in electrostatic repulsion between the two surfaces. Surface appendages such as pili already present on microbes or induced by the presence of a plant surface or other favorable conditions are used to bridge the gap exerted by the electrostatic repulsion *(31).* Bacteria colonize surfaces and are able to migrate by responding to changes within their growth environment *(17).* A wide variety of techniques have been used to study surface characteristics of bacterial cells *(6, 11, 21, 26–28, 32, 35, 41).* The most widely used techniques are hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC), because bacterial cell surface properties can be measured only indirectly, through phenomena that reflect more or less the nature of molecular interactions *(25).*

A better understanding of bacterial adhesion to the cantaloupe is necessary for the development of more effective washing treatments to control microorganisms on melon surfaces and fresh-cut pieces. The aim of this study was to determine the bacterial cell surface characteristics (surface charge and hydrophobicity) of *Listeria monocytogenes, Salmonella,* and *Escherichia coli* and to evaluate whether these characteristics are correlated with the strength of bacterial attachment to the outer surfaces of cantaloupes.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and preparation. Bacterial strains used in this study were *E. coli* ATCC 25922 (type strain), *E. coli* O157:H7 strains SEA13B88 and Oklahoma (apple juice cider-related outbreaks), *Salmonella* Stanley H0558 (alfalfa sprout–related outbreak, obtained from Dr. Patricia Griffin, Centers for Disease Control), *Salmonella* Poona RM2350, *Salmonella* Saphra 97A3312 (cantaloupe-related outbreaks, obtained from Ms. Sharon Abbott and Dr. Michael Janda, California Department of Health Services), *L. monocytogenes* Scott A (clinical isolate), *L. monocytogenes* CCR1-L-G (food isolate), *L. monocytogenes*

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ATCC 15313 (type strain), and *L. monocytogenes* H7888 (food isolate). Except where designated, bacterial strains were obtained from the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center culture collection.

Bacteria were maintained on brain heart infusion agar (Difco, Detroit, Mich.) slants held at 4°C. Prior to use, the cultures were subjected to two successive transfers by loop inocula to 5 ml of brain heart infusion broth (Difco) (*Salmonella* and *E. coli*) or 5 ml of Trypticase soy broth supplemented with 0.6% yeast extract (TSBYE; Difco) (*L. monocytogenes*). A final transfer of 0.2 ml was made into 20 ml of brain heart infusion broth or TSBYE, and incubation was carried out at 36°C for 18 h under static conditions. The bacterial cells were harvested by centrifugation (10,000 × *g*, 10 min) at 4°C. The cell pellets were washed in salt peptone (0.85% NaCl, 0.05% Bacto Peptone [Difco]). The cell pellets were used to prepare three different types of inoculum. The first type of inoculum consisted of the individual bacterial strains at 10^8 CFU/ml. The second type of inoculum consisted of a mixture containing strains of individual genera (three to four strains per genus). The final concentrations of *Salmonella, E. coli,* and *L. monocytogenes* in this inoculum were 1.23×10^8, 2.26×10^8, and 2.35×10^8 CFU/ml, respectively. Similar bacterial suspensions were also used for the chromatographic assay described below. The third type of inoculum consisted of a mixture containing 2.13×10^8 CFU of *Salmonella* Poona RM2350 per ml, 2.30×10^8 CFU of *E. coli* O157:H7 SEA13B88 per ml, and 1.97×10^8 CFU of *L. monocytogenes* Scott A per ml. All inocula were prepared in 3 liters of 0.1% (wt/vol) peptone water.

Chromatography. HIC and ESIC columns were prepared according to a slightly modified version of the procedure described by Dahlback et al. *(6)* and Pedersen *(27)*. Pasteur capillary pipettes (14.59 cm long; Macalaster Bicknell Co., Millville, N.J.), were plugged with glass wool and washed sequentially with 5 ml of 75% ethanol and 10 ml of 0.02 M sodium phosphate ($NaPO_4$) buffer (pH 6.8). Columns for HIC were packed with 8 ml of octyl-Sepharose CL-4B gel (Sigma Chemical Co., St. Louis, Mo.) equilibrated overnight at 4°C in 12 ml of 0.02 M $NaPO_4$ buffer (pH 6.8) (bed volume, 0.6 ml). The ESIC columns were packed with 2 ml of a 1:2 (wt/vol) suspension of the ion exchange resin and $NaPO_4$ buffer (bed volume, 0.6 ml). Dowex chloride form (1.2 meq/ml capacity; Sigma) was used for the anionic resin, and Dowex hydrogen form (1.7 meq/ml capacity, 50 × 8; Bio-Rad Laboratories, Richmond, Calif.) was used for the cation resin. The mesh size was 100 to 200 μm for both resins. Chromatography was carried out according to Dickson and Koohmaraie *(9)*. A sample (0.1 ml) of washed bacterial cell suspension of an individual strain was loaded onto the surface of the column, and then 0.2 ml of $NaPO_4$ buffer was added. The bacterial cell populations in the suspensions added to the column were determined by standard dilution plating techniques. The elution of all columns was performed with 12 ml of 0.02 M $NaPO_4$ buffer (pH 6.8), and the eluate was collected. Bacterial populations in each elute sample and in the original suspensions were determined with brain heart infusion agar or tryptic soy agar (Difco) by a pour plate technique. The number of bacteria bound to the columns was calculated as the difference between the total cell population in the initial suspension and that in the eluted sample. The relative hydrophobicity was expressed as *g/e,* and the relative ion values were expressed as *r/e,* with *g* and *r* being the numbers of bacteria retained by the columns and *e* being the number eluted. Each strain was tested three times.

Attachment experiments. Unwaxed Western "shipper" cantaloupes (1,461.5 to 1,948.1 g) obtained from a local distribution warehouse were allowed to come to room temperature (~20°C) overnight before being inoculated. Cantaloupes were submerged in 3 liters of bacterial inoculum (individual strains or cocktails) and agitated by stirring with a glove-covered hand for 10 min to ensure even inoculation. The inoculated cantaloupes were air dried for 1 h in a biosafety cabinet and then stored at 4°C for up to 7 days before washing treatments were applied. At 0, 3, and 7 days postinoculation, three cantaloupes per bacterial stain or cocktail were washed with water by submersion under the surface of 3 liters of sterile tap water, and then manual rotation was performed to assure complete coverage and contact of the surfaces with the wash solution for 2 min to remove loosely attached bacteria. Washed melons were placed on crystallizing dishes in a biosafety cabinet to dry for 1 h. Bacterial cells in the wash water and those remaining on the melon surfaces were enumerated as described below. The population remaining on the melon surface after the washing treatment was expressed in terms of the S_R value. The S_R value represents the percentage of the total bacterial population strongly attached to the cantaloupe. S_R values were calculated as (strongly attached bacteria)/(loosely attached bacteria + strongly attached bacteria) as described by Dickson and Koohmaraie *(9)*.

Microbiological examination. Plugs (2.2 cm; $n = 40$) weighing approximately 25 g altogether were cut from each cantaloupe rind with a sterile stainless steel cork borer and blended (Waring commercial blender, speed 5, 1 min) in 75 ml of 0.1% peptone water. *Salmonella* cells were enumerated on salmonella-shigella agar (Difco) with incubation at 35°C for 48 h. For comparison, a pure culture of *Salmonella* was plated on salmonella-shigella agar (Difco), incubated as above, and run in parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* colonies according to the *FDA Bacteriological Analytical Manual* following conventional biochemical methods *(1)* as well as serological assays involving latex agglutination (Oxoid, Ogdensburg, N.Y.).

For the enumeration of *E. coli,* plating was carried out on violet red bile agar (Difco) with a 5-ml overlay of the same agar containing 4-methylumbelliferyl-β-D-glucuronide, and plates were incubated at 37°C for 24 h. Selected colonies were confirmed to be *E. coli* colonies as described by Hitchins et al. *(18)*. In addition, for *E. coli* O157:H7, serological assays involving latex agglutination (Oxoid) were employed.

To enumerate *L. monocytogenes,* plating was carried out with modified Oxford agar (Difco) incubated at 37°C for 48 h *(20)*. In addition, pure cultures of *L. monocytogenes* were surface plated onto modified Oxford agar to serve as references for identification. Representative presumptive colonies of *L. monocytogenes* were subjected to API *Listeria* tests (bioMerieux Marcy l'Etiole, France).

RESULTS

Relative bacterial cell surface hydrophobicity and charge. Bacterial surface hydrophobicity showed substantial variation among strains of *Salmonella* but not among strains of *E. coli* or *L. monocytogenes* (Table 1). The highest cell surface hydrophobicity was exhibited by the *Salmonella* strains (*g/e* = 0.338 to 0.629), followed by *L. monocytogenes* and then *E. coli.* There was no difference between the surface hydrophobicity of *E. coli* ATCC 25922 and that of the *E. coli* O157:H7 strains. The hydrophobicity values for the *L. monocytogenes* strains were also very similar.

All of the bacteria tested exhibited stronger negative

TABLE 1. *Bacterial cell surface hydrophobicity and charge*[a]

Bacterium	Hydrophobicity (*g/e*)	Surface charge (*r/e*)	
		ESIC (−)	ESIC (+)
Salmonella			
Stanley (H0558)	0.338 ± 0.114	21.48 ± 0.19	4.10 ± 0.10
Poona (RM2350)	0.486 ± 0.110	33.71 ± 0.30	1.82 ± 0.14
Saphra (97A3312)	0.629 ± 0.130	50.00 ± 0.15	6.08 ± 0.11
Escherichia coli			
ATCC 25922	0.233 ± 0.021	1.62 ± 0.12	0.12 ± 0.04
O157:H7 SEA13B88	0.207 ± 0.015	1.48 ± 0.10	0.18 ± 0.09
O157:H7 Oklahoma	0.220 ± 0.019	1.50 ± 0.13	0.16 ± 0.03
Listeria monocytogenes			
Scott A	0.284 ± 0.051	38.06 ± 0.12	0.40 ± 0.12
ATCC 15313	0.278 ± 0.029	38.11 ± 0.10	0.32 ± 0.08
CCR1-L-G	0.282 ± 0.059	37.68 ± 0.14	0.20 ± 0.04
H7778	0.280 ± 0.46	37.47 ± 0.12	0.08 ± 0.04

[a] Values are averages of three separate determinations ± standard deviation.

than positive surface charges (Table 1). *Salmonella* Saphra had the strongest negative surface charge, with an *r/e* value of 50. The other two *Salmonella* strains and the four *L. monocytogenes* strains had values ranging from 21 to 38. The three strains of *E. coli* had the weakest negative surface charge. The surface charges of *E. coli* ATCC 25922 and the *E. coli* O157:H7 strains were similar. There were no differences between the surface charges of *L. monocytogenes* strains. The strains of *E. coli* and *L. monocytogenes* had similar weak positive surface charges compared with that of the *Salmonella* strains. The overall total charge was very close for bacterial strains within a genus except for *Salmonella.*

Attachment to cantaloupe surfaces and S_R value. The number of *E. coli* cells attached to the cantaloupe surface was larger than that for *Salmonella* or *L. monocytogenes* (Table 2). The population of *E. coli* on the cantaloupe surface ranged from 5.20 to 5.81 $\log_{10}$ CFU/cm^2, compared with 4.34 to 4.84 $\log_{10}$ CFU/cm^2 for *Salmonella* and 2.89 to 3.20 $\log_{10}$ CFU/cm^2 for *L. monocytogenes.* Although the *E. coli* strains exhibited the largest initial numbers of attached bacteria on the melon surface, the highest S_R value was that for *Salmonella,* followed by *L. monocytogenes* and then *E. coli.* Higher S_R values indicate stronger bacterial attachment to the melon surface, as indicated by the relative inability of washing treatments to detach the pathogen from the melon surface with water.

TABLE 2. *Bacterial attachment on melon surfaces in relation to* S_R *on day 0*[a]

Bacterium	$\log_{10}$ CFU/cm^2	S_R
Salmonella		
Stanley H0558	4.84 ± 0.10	0.920 ± 0.009
Poona RM2350	4.37 ± 0.11	0.939 ± 0.010
Saphra 97A3312	4.34 ± 0.18	0.942 ± 0.011
Escherichia coli		
ATCC 25922	5.53 ± 0.15	0.763 ± 0.052
O157:H7 SEA 13B88	5.81 ± 0.21	0.750 ± 0.041
O157:H7 Oklahoma	5.20 ± 0.18	0.739 ± 0.059
Listeria monocytogenes		
Scott A	2.89 ± 0.09	0.826 ± 0.038
ATCC 15313	3.00 ± 0.10	0.798 ± 0.032
CCR1-L-G	3.12 ± 0.11	0.830 ± 0.021
H7778	3.20 ± 0.09	0.810 ± 0.051

[a] Inocula consisted of individual bacterial strains; the S_R value represents the strength of attachment.

Results of a study examining the attachment of bacteria from mixed cocktails containing all strains of an individual genus (*Salmonella, E. coli,* or *L. monocytogenes*) on the surfaces of cantaloupes stored at 4°C for up to 7 days are shown in Table 3. Again, *Salmonella* had the highest S_R value, indicating stronger attachment to the cantaloupe surface either as individual strains or as a mixed cocktail. The S_R value for *Salmonella* decreased from 0.925 to 0.902 by day 3 of storage at 4°C but increased to 0.949 by day 7. The strength of attachment for *E. coli* increased slightly over the 7 days of storage, but that for *L. monocytogenes* decreased.

When *E. coli* O157:H7 SEA13B88, *Salmonella* Poona RM2350, and *L. monocytogenes* Scott A were mixed and used as a cocktail to inoculate cantaloupe surfaces, the S_R value for *Salmonella* was again higher than that for *E. coli* or *L. monocytogenes* on all three storage days, indicating stronger attachment (Table 4). Also, the S_R value for *Salmonella* Poona increased once again (from 0.828 to 0.923) over the 7-day period of storage at 4°C.

Correlation of bacterial cell surface hydrophobicity and charge with attachment to cantaloupe rind. The correlation coefficients for cell surface hydrophobicity and attachment to the cantaloupe surface and for cell surface charge and attachment to the cantaloupe surface for the individual cocktails of the three genera for day 7 are shown in Table 5. The data suggest a linear relationship between bacterial attachment and both bacterial cell surface charge

TABLE 3. *Strength of bacterial attachment to cantaloupe surfaces for inoculum cocktails containing strains of a single genus*

Bacteria	S_R values on day of storage at 4°C		
	0	3	7
Salmonella[a]	0.925 ± 0.025	0.902 ± 0.110	0.949 ± 0.102
E. coli[b]	0.760 ± 0.113	0.787 ± 0.052	0.807 ± 0.094
L. monocytogenes[c]	0.717 ± 0.109	0.663 ± 0.064	0.659 ± 0.089

[a] Cocktail of *Salmonella* spp. containing 1.23×10^8 CFU of *Salmonella* Stanley H0558 per ml, 2.20×10^8 CFU of *Salmonella* Poona RM2350 per ml, and 1.16×10^8 CFU of *Salmonella* Saphra 97A3312 per ml.

[b] Cocktail of *E. coli* containing 2.18×10^8 CFU of *E. coli* ATCC 25922 per ml, 2.30×10^8 CFU of O157:H7 SEA13B88 per ml, and 2.26×10^8 CFU of O157.H7 Oklahoma per ml.

[c] Cocktail of *L. monocytogenes* containing 2.08×10^8 CFU of strain Scott A per ml 2.35×10^8 CFU of strain ATCC 15313 per ml, 2.10×10^8 CFU of strain LM-4 per ml, and 2.21×10^8 CFU of strain H7778 per ml.

and hydrophobicity. For the *Salmonella* and the *E. coli* cocktails, correlation coefficients for positive surface charge and attachment to the melon surface were higher than those for negative surface charge and attachment. The *E. coli* cocktail showed the highest correlation coefficient for cell surface hydrophobicity. The cocktail of *L. monocytogenes* strains showed similar correlation coefficients for negative and positive cell surface charge and hydrophobicity. The data suggest that both surface hydrophobicity and surface charge play major roles in bacterial attachment to cantaloupe surfaces.

When the values for all 10 individual bacterial strains were used to determine the relationship between bacterial cell surface hydrophobicity and the strength of attachment (S_R) on day 0, a linear correlation coefficient of 0.767 was observed (Fig. 1). The correlation coefficients for the relationship between the relative negative and positive charges on the bacteria and their attachment to the cantaloupe surface ($r^2 = 0.738$ and 0.724, respectively) were similar to those for hydrophobicity (Figs. 2 and 3). This finding again suggests that both hydrophobicity and charge play a role in the attachment of the pathogens to the surface of the cantaloupe rind.

DISCUSSION

Bacterial surfaces are heterogeneous, and their physicochemical properties are determined primarily by teichoic acid (gram-positive strains) or other polysaccharides (gram-negative strains) along with proteinaceous appendages (fimbriae) *(12, 28, 30).* This heterogeneity may help to explain the differences observed in bacterial hydrophobicity or cell surface charge in relation to bacterial attachment to the cantaloupe surface. The present study is the first in which HIC and ESIC techniques were used to investigate the relationship between the cell surface charge and the hydrophobicity of *Salmonella, E. coli,* and *L. monocytogenes* and their strength of attachment to the cantaloupe rind surface. The results of our HIC and ESIC studies are similar to those of Dickson and Koohmaraie *(9),* who used phenyl-Sepharose CL-4B gel (less hydrophobic than the octyl-Sepharose CL-4B used in this study) for HIC and Dowex resins (chloride and hydrogen form) for ESIC. Dickson and Koohmaraie reported *g/e* values of 0.392 for *Salmonella* Typhimurium ATCC 14028, 0.203 for *E. coli* O157:H7 (no strain designation given), and 0.278 for *L. monocytogenes* Scott A. Negative *r/e* values reported for *Salmonella, E. coli,* and *Listeria* were 9.47, 1.33, and 37.73, respectively, and positive *r/e* values were 4.78, 0.16, and −0.44, respectively. Increased attachment to fatty beef tissue was highly correlated with both increased bacterial cell surface negative charge and hydrophobicity, but attachment to lean beef tissue was highly correlated only with relative negative charge *(9).*

The outer surface (rind) of a cantaloupe presents a variety of surfaces to which a bacterium may bind. The epidermal cell surface is ruptured with a meshwork of raised tissue (the net). This net consists of lenticels and phellum (cork) cells. These cells have hydrophobic suberized walls to reduce water loss and protect against pathogen ingress. Also imparting a hydrophobic nature to the outer surface of the cantaloupe is the cuticle, composed of waxes and cutin, that covers the epidermal cells *(44).* Hydrophilic components of plant cell walls and middle lamella may also be exposed because of cuticular cracks and injuries to the epidermal surface. The mechanism of attachment of bacterial cells to plant surfaces has been studied most exten-

TABLE 4. *Strength of bacterial attachment to cantaloupe rinds for an inoculum cocktail containing three bacterial strains*[a]

Bacterium	S_R value on day of storage at 4°C		
	0	3	7
Salmonella Poona RM2350	0.828 ± 0.051	0.917 ± 0.114	0.923 ± 0.071
E. coli O157:H7 SEA13B88	0.746 ± 0.110	0.800 ± 0.060	0.806 ± 0.110
L. monocytogenes Scott A	0.628 ± 0.038	0.721 ± 0.049	0.716 ± 0.013

[a] The cocktail contained a mixture of *Salmonella* Poona RM2350 (2.13×10^8 CFU/ml), *E. coli* O157:H7 SEA13B88 (2.30×10^8 CFU/ml), and *L. monocytogenes* Scott A (1.97×10^8 CFU/ml).

TABLE 5. *Correlation coefficients for bacterial cell surface hydrophobicity and charge and strength of attachment to cantaloupe surfaces on day 7*

Bacteria[a]	Correlation coefficient		
	Surface charge (r/e)		Hydrophobicity (g/e)
	ESIC (−)	ESIC (+)	(HIC)
Salmonella cocktail	0.787	0.878	0.857
E. coli cocktail	0.887	0.944	0.998
L. monocytogenes cocktail	0.995	0.984	0.956

[a] The compositions of the bacterial cocktails were as described in Table 3.

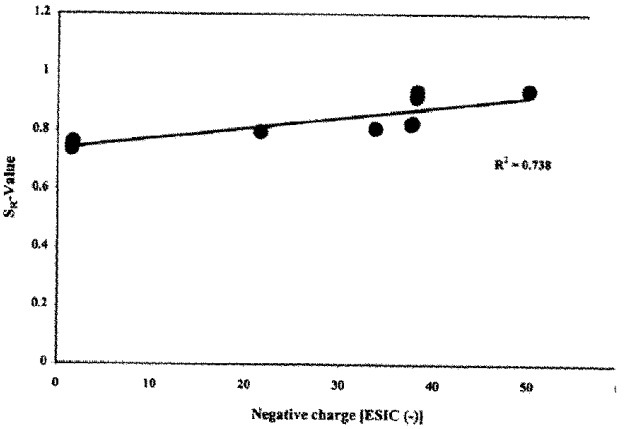
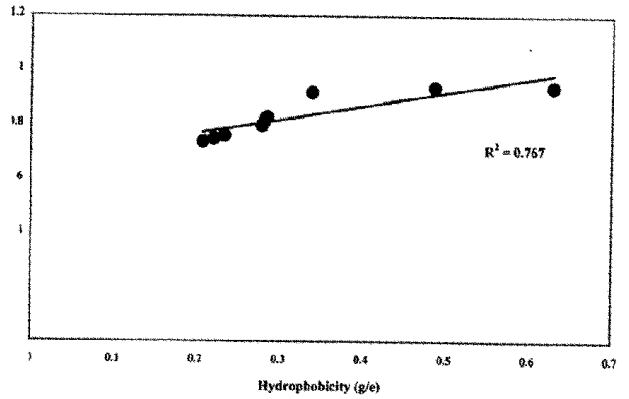


FIGURE 2. *Relationship between bacterial surface negativ charge (ESIC (−)) of individual bacterial strains and attachmen to cantaloupe surfaces on day 0.*

sively for plant pathogens and symbionts *(30, 31)*. Flagella, fimbriae, outer membrane proteins, and extracellular polysaccharides have all been implicated in bacterial attachment. In contrast, there is little information available on the attachment of bacterial human pathogens to plant surfaces. Studies involving confocal scanning laser microscopy indicate that *Salmonella, E. coli* O157:H7, and *L. monocytogenes* can attach to intact plant surfaces such as trichomes and can be present in substomatal chambers but are found most often on wounded surfaces and within cuticular cracks *(5, 19, 33, 37, 38)*.

The results of the present study indicate that surface hydrophobicity and both negative and positive charges are highly correlated with the strength of attachment of *Salmonella, E. coli* O157:H7, and *L. monocytogenes* to the cantaloupe rind. However, because of the wider variation in cell surface hydrophobicity among *Salmonella* serovars, additional serovars should be examined. That all three of these surface properties affect the attachment of bacteria to the plant surface could be predicted from the heterogeneous nature of a cantaloupe surface, as discussed above. The intact surface of a cantaloupe is hydrophobic in nature, and *Salmonella,* which has the most hydrophobic surface of the hree genera tested, bound the strongest (i.e., had the high-st S_R values) on each testing day. Recently, *Salmonella* vas demonstrated to produce the extracellular carbohydrate olymer cellulose, and cellulose and curli (aggregative fim-



RE 1. *Relationship between bacterial cell surface hydrocities of individual strains and attachment to cantaloupe es.* S_R *values represent the strength of bacterial attachment 0.*

briae), as the two principle components of the extracellular matrix, are thought to be responsible for biofilm formation *(4, 45)*. Interestingly, for the plant pathogen *Agrobacterium* as well as the plant symbiont *Rhizobium,* cellulose production plays an important role in the firm attachment of bacteria to plants and in the formation of bacterial aggregates at the plant surface *(30)*. After initial attachment, *Agrobacterium* synthesizes cellulose fibrils that bind this bacterium very tightly to the host cell surface and to each other, and the bacterial cells can be removed only by digestion of the bacterial or host cell wall *(23)*. Fibrils of an unknown nature have been observed for *Pseudomonas putida* and *Pseudomonas tolaasi* binding to the surface of the *Agaricus bisporus* mycelium *(29)*, for *Pseudomonas syringae* pv. *syringae* binding to apple tissues *(22)*, and for *Azospirillum brasilense* binding to tomato, cotton, and pepper roots *(2)*.

Cellulose production and the presence of curli may allow for the strong attachment of *Salmonella* to the cantaloupe rind. The growth temperature used for the preparation of the inoculum in our experiments (36°C) does not favor the production of these two surface components, as temperatures above 26 to 28°C repress their production *(45)*. The production of these surface components by *Salmonella* during storage of inoculated cantaloupe would lead to an increased binding strength, as was seen over the 7-day stor-

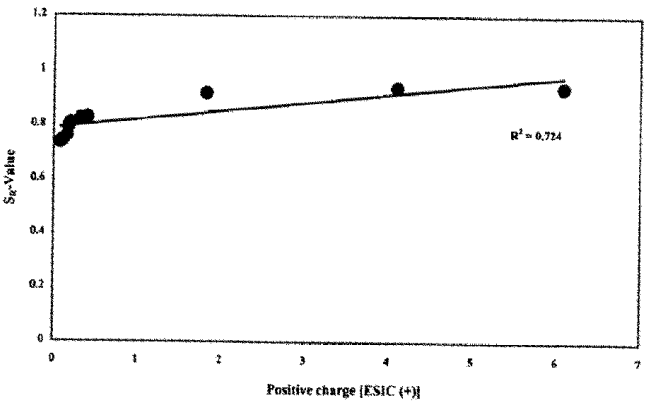


FIGURE 3. *Relationship between bacterial surface positive charge (ESIC (+)) of individual bacterial strains and attachment to cantaloupe surfaces on day 0.*

age period, but these surface components may not be produced at a storage temperature of 4°C. Under natural conditions, when contamination occurs in the field, the production of cellulose and curli by *Salmonella* may allow the bacterium to strongly bind to the plant surface and to be highly resistant to removal by rain or by washing during processing *(39, 40)*. Some strains of *E. coli* O157:H7 are also known to produce fimbriae and bacterial exopolysaccharides *(16, 34)*, and these strains may also function as plant surface adhesins. It is difficult to predict the surface properties of bacterial human pathogens when the pathogens are first exposed to a plant surface, as environmental conditions can significantly affect bacterial surface properties, including charge and hydrophobicity *(3, 7, 34)*.

The results of this study indicate that both charge and hydrophobicity influence the attachment of bacterial human pathogens to cantaloupe rinds. Bacterial cell surface hydrophobicity was identified as an important factor in bacterial attachment and immobilization in the intercellular spaces of soybean leaves *(10)*. However, De Wegner et al. *(8)* reported that neither surface hydrophobicity nor charge determines the ability of *Pseudomonas* species to adhere to plant roots. Specific interactions between complementary moieties such as bacterial carbohydrate polymers and plant lectins or fimbriae and plant carbohydrate-containing moieties may also play a role *(24)*, especially in bacterial attachment to exposed plant cell wall materials and damaged tissues.

This study represents a first step in obtaining a better understanding of how bacterial human pathogens attach to plant surfaces. Future studies will include an investigation of the effect of environmental stresses such as cold storage on the surface characteristics of bacterial pathogens and their attachment to cantaloupe surfaces. The relationship of bacterial surface characteristics to the attachment of bacteria to other types of produce will also be investigated. The knowledge gained from such studies will allow for the development of much-needed improved intervention strategies to help insure the microbial safety of produce.

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